The UV-Protective Potential of Ethanolic Extract of Sukun (Artocarpus Altilis (Park.) Fosberg) Leaves: In Vitro and In Vivo Study

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Abstract

Sukun (Artocarpus altilis (Park.) Fosberg) leaves contain bioactive flavonoids with significant photoprotective potential. This study aimed to evaluate the UV-protective activity of the ethanolic extract of Sukun leaves using in vitro and in vivo methods. The in vitro assay was performed with UV–Visible spectrophotometry, and the Sun Protection Factor (SPF) value was calculated using the modified Mansur equation. The in vivo study used a post-test only control group design with 24 male Wistar rats divided into five groups: ethanolic extract concentrations of 30%, 40%, and 50%, a positive control (sunscreen containing benzophenone-3 and octyl methoxycinnamate), and a negative control (distilled water). Erythema scores were recorded and analyzed using SPSS 18. The results demonstrated that the ethanolic extract at concentrations of 30%, 40%, and 50% exhibited SPF values of 34.00, 35.76, and 36.54, respectively—classified as ultra-protection levels comparable to the synthetic sunscreen control (SPF 33). In vivo, erythema scores of 2, 0, and 0, respectively, indicated strong photoprotective activity similar to the positive control. These findings suggest that Artocarpus altilis Leaves *extract has promising potential as a natural UV-protective ingredient in sunscreen formulations.*

Introduction

Ultraviolet (UV) radiation from sunlight reaching the Earth's surface is categorized into UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm). Among these, UVB contributes most to erythema, oxidative stress, and photoaging (Krutmann et al., 2023). Excessive exposure to ultraviolet (UV) radiation is associated with various adverse skin effects, including erythema, photoaging, oxidative damage, and an increased risk of skin cancer, which highlights the importance of developing safe and effective UV-protective agents from natural sources (Nigel et al., 2023). Modern sunscreen formulations combine synthetic and mineral UV filters designed to provide broad-spectrum protection against UVA and UVB radiation. Verma et al report that recent advances in sunscreen technology also incorporate natural phytochemicals as active ingredients to enhance UV absorption, stabilize synthetic filters, and strengthen antioxidant defense. This approach reflects a shift from earlier sunscreen standards toward more effective and comprehensive formulations (Verma et al., 2024).

According to Fonseca et al (2023), flavonoids and other phenolic compounds can act as natural sunscreen agents because their conjugated aromatic structures enable the absorption of UV radiation, while their strong antioxidant properties allow them to neutralize reactive oxygen species. he Artocarpus genus is known for its wide spectrum of bioactive phytochemicals and pharmacological properties (Fonseca et al., 2023). Specifically, Artocarpus altilis leaves have been reported to contain various flavonoid compounds, including flavonoids and phenolic constituents, which contribute to their antioxidant and potential UV-protective activities (Ulvia et al., 2024) (Nirwana Sembiring et al., 2023).

This study aimed to assess the UV-protective activity of the ethanolic extract of Sukun leaves both in vitro and in vivo to support its application as a natural photoprotective compound.

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Materials and Methods

The laboratory equipment used in this study included a measuring cup (HARIO CMJW-500, HARIO, Japan), an evaporating dish (Pyrex, Corning, USA), a stainless steel sieve (30 mesh, Endecotts Ltd., UK), and a blender (Philips HR2221, Philips, Indonesia). Quantitative measurements were performed using an analytical balance (OHAUS Pioneer PA214, OHAUS Corporation, USA). Spectrophotometric analysis was conducted with a UV-Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan) equipped with optical cuvettes (BrandTech, Germany), and all glassware (DURAN®, Schott AG, Germany) was made of borosilicate glass. The UV-B lamp (Philips TL-20W/12, Philips, Netherlands) served as the radiation source for in vivo testing, and animal cages (Tecniplast, Italy) were used for housing the Wistar rats. A custom-made exposure box (local manufacturer, Semarang, Indonesia) ensured controlled UV exposure, and aluminum foil (Reynolds®, Reynolds Consumer Products, USA) was applied to protect unexposed skin areas.

The materials used in this study included Sukun leaves collected from Ungaran, Central Java, Indonesia; distilled water (OneMed, Indonesia); ethanol 70% and 96% (Merck KGaA, Darmstadt, Germany); methanol (Merck KGaA, Darmstadt, Germany); sulfuric acid (H_2SO_4, Merck KGaA, Darmstadt, Germany); and ammonia solution (NH_3, Merck KGaA, Darmstadt, Germany). Synthetic sunscreen reference ingredients used were benzophenone-3 (Sigma-Aldrich, USA) and octyl methoxycinnamate (Sigma-Aldrich, USA).

Plant Determination

Plant identification was performed at the Ecology and Biosystematics Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, and confirmed according to taxonomic descriptions of Artocarpus altilis (Lathiff et al., 2021).

In Vitro Analysis

Extract Preparation

Fresh leaves were washed, air-dried under shade, powdered, and sieved. The powdered leaves were macerated in 70% ethanol, and the filtrate was evaporated at 60°C to obtain a thick ethanolic extract.

Flavonoid Identification

Flavonoid content was confirmed using colorimetric tests, where color changes from yellowish-green to red (methanol + H_2SO_4) and to yellow (ammonia + concentrated acid) indicated flavonoid presence, consistent with findings by (Ghaisani Yumni et al., 2024).

In Vitro SPF Determination

UV–Visible spectrophotometry was employed to determine SPF values of the ethanolic extracts (30%, 40%, and 50%). The SPF value was calculated using the modified Mansur equation (Sharma et al., 2020) (El-otmani et al., 2024).

$$SPF = CF x \sum_{290}^{320} EE\ (\gamma)\ x\ I\ x\ abs\ (\gamma)$$

description:
SPF = Sun Protection Factor
CF = Correction Factor (10)
EE = Erythema effect spectrum
I = Radiation spectrum intensity
Abs = Test sample absorbance

In Vivo Erythema Analysis

The anti-inflammatory potential of the test compound was assessed using an in vivo UV-B-induced erythema model in rats. Male Wistar rats were randomly assigned to five groups: negative control (distilled water), positive control (synthetic sunscreen containing benzophenone-3 and octyl methoxycinnamate), and three treatment groups receiving ethanolic extracts of Artocarpus altilis leaves at concentrations of 30%, 40%, and 50%. Each treatment was applied topically to the dorsal skin for approximately one hour, followed by exposure to UV-B radiation for 200 minutes. Erythema was evaluated visually one hour post-exposure, and scores were recorded to determine the anti-inflammatory and photoprotective effects of the treatments. (Yang et al., 2015) . Erythema Score Determination based on (Mayangsari et al., 2024).

Score 0 = no erythema
Score 1 = very slight redness

Score 2 = clearly defined erythema
Score 3 = moderate to severe erythema
Score 4 = severe redness (beet red) to slight crust formation (deep wound).

Statistical analysis was conducted using SPSS version 18.0 (IBM Corp., USA), employing the Mann–Whitney U test to determine significant differences between treatment groups, with $p < 0.05$ considered statistically significant.

Results and Discussion

The plant determination of Sukun *leaves* (Artocarpus altilis (Park) Fosberg) classification details were recorded. It was determined to belong to Famili 117. Moraceae, Genus 9. Artocarpus, and Species Artocarpus altilis (Park) Fosberg. The plant determination was performed to ensure the accuracy of the plant material used and to minimize the risk of misidentification, which is critical in studies involving pharmacological testing. Accurate taxonomic identification is a fundamental requirement to validate the relevance and reliability of subsequent experimental results. Based on the determination analysis, the plant sample utilized in this study was confirmed to be Sukun *leaves* (Artocarpus altilis (Park.) Fosberg). This confirmation supports the validity of the findings obtained from the pharmacological evaluations conducted.

The results of flavonoid identification in the ethanol extract of Sukun leaves showed a color change from yellowish-green to red upon the addition of methanol and H_2SO_4. Furthermore, a color change to yellow occurred upon the addition of dilute ammonia and concentrated sulfuric acid. This demonstrates the presence of flavonoid compounds in the Sukun leaves.

Table 1. Flavonoid Identification Results

Compound Group	Flavonoid Test	Color Change
Flavonoid	Sukun leaves extract + methanol heated + H_2SO_4	Yellowish-green → red (chalcone)
Flavonoid	Sukun leaves extract + dilute NaOH + H_2SO_4	Yellowish-green→yellow (flavonoid)

In Vitro Analysis The in vitro UV protection activity test of Sukun leaves ethanol extract yielded SPF values of 34.00 for the 30% concentration, 35.76 for the 40% concentration, and 36.54 for the 50% concentration. These values correspond to the ultra protection category according to FDA protection classification. The data shows that the greater the concentration of an extract, the greater its SPF value. The SPF values obtained for the Sukun leaves extract at 30%, 40%, and 50% concentrations are greater when compared to the positive control (Benzophenone-3 and octyl-methoxicinnamate), which had an SPF value of 33. Sunscreens with SPF 30 are known to absorb 97% of UV-B radiation. The substantial flavonoid content in the Sukun leavescontributed to the high SPF values found in the ethanol extracts at all tested concentrations.

Table 2. Results of SPF Value Calculation and Protection Type

Extract	SPF Value	Protection Type
Concentration 30%	34.00	Ultra
Concentration 40%	35.76	Ultra
Concentration 50%	36.54	Ultra

In Vivo Analysis Observation of erythema scores, based on the mode (most frequent score) in each treatment group, indicated that the 30% extract had an erythema score of 2. The 40% concentration extract, the 50% concentration extract, and the positive control all had an erythema score mode of 0. The negative control had an erythema score of 4.

Table 3. Erythema Score

Treatment Group	Erythema Score
Concentration 30%	2
Concentration 40%	0
Concentration 50%	0
Negative Control	4
Positive Control	0

Statistical analysis was performed using SPSS 18 (IBM Corp., USA) at a 95% confidence level employing the Mann–Whitney U test to determine significant differences between treatment groups, with $p < 0.05$ considered statistically significant. The Shapiro–Wilk normality test indicated $P < 0.05$, showing that the data were not normally distributed, while the homogeneity test yielded $P = 0.457$, indicating that the data were homogeneous. Since the data were not normally distributed but homogeneous, the Kruskal–Wallis test was applied, resulting in $P = 0.014$, which demonstrated a significant difference among the treatment groups in terms of erythema scores.

Table 4. Mann-Whitney Test Results

Treatment Group	P-value	Conclusion
Concentration 30% vs 40%	0.046	Significantly different
Concentration 30% vs 50%	0.119	Not significantly different
Concentration 30% vs negative control	0.034	Significantly different
Concentration 30% vs positive control	0.653	Not significantly different
Concentration 40% vs Concentration 50%	0.317	Not significantly different
Concentration 40% vs negative control	0.011	Significantly different
Concentration 40% vs positive control	0.131	Not significantly different
Concentration 50% vs negative control	0.015	Significantly different
Concentration 50% vs positive control	0.405	Not significantly different
Negative control vs positive control	0.037	Significantly different

Subsequent Mann–Whitney tests revealed that all extract concentrations (30%, 40%, and 50%) were significantly different from the negative control ($P < 0.05$), indicating that distilled water alone did not provide UV protection, whereas the extract exhibited protective activity.

Comparisons with the positive control showed no significant differences ($P > 0.05$), suggesting that the extract's UV protection effect was comparable to the commercial sunscreen. Among the extract concentrations, 30% vs 40% showed a significant difference ($P = 0.046$), indicating that increasing the concentration from 30% to 40% enhanced UV protection, while 30% vs 50% and 40% vs 50% were not significantly different ($P = 0.119$ and $P = 0.317$, respectively). suggesting that further increases beyond 40% did not significantly improve efficacy. These findings indicate that the extract exhibits a dose-dependent effect up to 40%, after which the protective effect plateaus.

Conclusion and Suggestion

The ethanol extract of Sukun leaves (Artocarpus altilis (Park) Fosberg) has UV protection activity in vitro and in vivo. The ethanol extract of Sukun leaves at optimum concentrations of 30%, 40%, and 50% has UV protection activity, providing SPF values of 34.00, 35.76, and 36.54, respectively, comparable to the positive control (benzophenone-3 and octyl methoxicinnamate) SPF 33 in vitro. The ethanol extract of Sukun leaves has UV protection activity at optimum concentrations of 30%, 40%, and 50%, seen from the mode of erythema scores being score 2, score 0, and score 0, respectively, in vivo. Future studies are recommended to explore the potential of Sukun leaves (Artocarpus altilis (Park.) Fosberg) as an active UV-protective agent, particularly its applicability in the development of sunscreen formulations.

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